RESEARCH REGARDING THE DENSITY OF THE MYOCYTES AND THE RATIO OF THE MAIN TISSUE CATEGORIES FROM THE LATERAL MUSCLE OF THE *POLYODON SPATHULA* SPECIES

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Abstract: *Histological samples were taken from the lateral muscle (dorsal epaxial) (ML-ED), from ten four year old sturgeon (Polyodon spathula) male specimens (Ps3+). These samples were processed with the paraffin sectioning technique and 20 slides with transversal sections of the lateral muscle were obtained. The slides where colored according to the HEA method. They were analyzed with an optic microscope (OM), type MC3. Both diameters (long and short) were measured in the microscopic field for the myocytes and the primary muscular fibers (PMB). Also in the microscopic field the myocytes from the PMB were counted. Through calculations, the average diameter, the circumference and the values for the shape and profile index were determined, at the PMB level. Afterwards the myocytes density and the ratio of the two tissue categories (muscle tissue and conjunctive tissue) at PMB and SMB level were determined. The following results were obtained: the average thickness of the PMB, from the 5 secondary muscle bundles (SMB) studied it varied between 318μ and 452.5μ, with a mean of 383.85μ. The average circumference of the PMB was 1206μ, the shape is a cylindroid with a flattening tendency (If=1,768/1)(Ip=59,19%). The PMB myocyte number oscillated between 34.2 and 72.33, the average being 50.76 muscle fibers. The myocyte density varied between 444.282fm/mm² and 489.166fm/mm², with a mean of 467.383fm/mm². The muscle tissue ratio varied between 64.10% and 75.0%, with a mean of 68.72%. The differences between the means of the five SMB, in accordance with the 11 studied parameters, were tested for statistical purposes, ascertaining that 99.09% of them are insignificant.*

Keywords: *Polyodon spathula; dorsal epaxial; myocytes; density; muscle tissue.*

INTRODUCTION

The rapid human population growth on our planet and the increase in quality and quantity demands regarding food, has determined the introduction of new animal species into consumption, as well as the improvement of their bio-economic performances. Apart from mammals and birds, humans also eat the meat and roe from different species of fish or other aquatic animals. In Romania, pisciculture has a very old tradition, our waters being known as highly productive in this field. Many fish species are bred in mono or mixed culture, ensuring large quantities of meat and roe for the food industry and for direct human consumption. Existing literature data specifies that 12-15% of our diet consists of fish and fish-based products (from the total of proteins consumed), with major differences according to geolocation. Fish meat is widely known for its distinct organoleptic properties; for its high digestibility rate and for its nutritional and superior biological value, due to the high content of proteins and essential aminoacids; the high percentage of unsaturated fats and the high quantity of vitamins and minerals. Sturgeons are among the oldest species to inhabit the waters of the Northern hemisphere and because their meat and roe (caviar) are in high

demand, it almost lead to the extinction of this taxonomic order. This is one of the many reasons that lead to breeding sturgeons in stock ponds (aquaculture). In Romania it began in 1992, when the Nucet research station, imported *Polydon spathula* larvae from the USA. In this article, some quality aspects of the *Polydon spathula* meat were studied, specifically the histological structure of the muscles, by analyzing a series of parameters at the myocytes level and muscle fibers from the lateral muscle (dorsal epaxial muscle).

MATERIALS AND METHODS

The manner in which our research took place demanded the use of several categories of materials (biological and nonbiological) and working methods. The biological material consisted of 10 four year old Polydon spathula males (Ps3+). The studied species has the following taxonomy: Kingdom - **Animalia**; Phylum - **Chordata**; Subphylum - **Vertebrata;** Class - **Actinopterygii**; Order - **Acipenseriformes;** Family - **Polyodontidae**; Genus – **Polyodon** (Lacepede - 1797); Species - P. **Spathula.** The Paddlefish (*Polyodon spathula*) is a sturgeon native to the Mississippi (from the Great Lakes to Florida)[2]. It is a large fish, reaching up to 1.5-2 meters in length when mature and with a body weight of 30-50-80 kg (in optimal feeding conditions). Paddlefish can live up to 30 years. The skin is smooth (with microscopic scales); lateral-oriented small eyes; large mouth, with a very long rostrum (one third of the body length) and flattened like a paddle or spatula. This rostrum is part of the cranium structure [2]. This species has two very important bio-economic advantages: it eats plankton (it feeds with zoo and phytoplankton and also with aquatic insects) and it has a rapid growth rhythm. In aquaculture it does not necessarily need supplemental feeding with concentrated fodder, although this aspect has been studied and applied in the US for several decades.

From the 10 sacrificed fish, histological samples were collected from the lateral muscles, more specifically from the dorsal epaxial muscles (ML-ED). These samples were processed according to the paraffin sectioning method [12][13]. Specific anatomy tools were used for the sample collection (forceps, scalpels, scissors, dissection needles, retractors, etc.). Twenty slides with transversal sections of the lateral muscle were obtained. The slides where colored according to the HEA method [12][13].They were analyzed with an optic microscope (OM), type MC3, which was calibrated in advance.The calibration was done for three types of eyepieces: OC10xOB6; OC10xOB10 and OC10xOB20. The micrometric values (MV) from the calibration of the MO-MC3 were: VM=15,000µ, for OC10xOB6; VM=9,011µ, for OC10xOB10 and VM=4,441µ, for OC10xOB20 [9][10]. For the study of the slides the following were also used: an eyepiece micrometer, a calibration blade, a myocyte numbering grate, a digital camera and other instruments. After the best images were identified in the microscopic field, the two diameters of the myocytes and of the primary muscle fascicles (PMF) were measured (big and small) and all myocytes from the PMF were counted. Through calculus the average diameter of the myocytes and PMF was determined; the perimeter of the muscle structures; the shape and profile indices; the transversal section surface of the myocytes and of the PMF, as well as the myocyte density. Also the ratio of the two main tissue categories, the muscle tissue and conjunctive tissue, was measured. For these measurements the following mathematical equations were used:

(1) Dx = (LD+Sd)/2, where: LD = large diameter of myocytes and of PMF, in microns (μ); Sd = small diameter of myocytes and of PMF (μ); Dx = average diameter (μ).

(2) P = (LD+Sd)/2x π, where: P = myocytes perimeter and of PMF (μ); π=calculation coefficient for the lenght and surface of the circle, with the value of 3,141592656.

(3) If = LD/Sd, where: If = format index of myocytes and of PMF (x/1).

(4) Ip = (Sdx100)/LD, where: Ip = Profile index of myocytes and of PMF (%).

(5) C.s.s. = [(DMxDM)/4]xπ, where: C.s.s. = cross-section surface of myocytesn and PMF (μ^2)

(6) Dmf = (nx1000000)/C.s.s.PMF, where: n = number of myocytes from PMF; Dmf = density of muscular fibers (myocytes) (mf/mm^2); C.s.s.PMF =cross-section surface of PMF (μ^2)

(7) MTP = (C.s.s.mf.x100)/ C.s.s.PMF, where: MTP = muscle tissue proportion (%); C.s.s.mf. = cross-section surface of the muscular fibers (μ^2)

(8) CTP = 100-MTP, where: CTP = connective tissue proportion (%).

All the data resulted from the micrometric measurements and from the calculus were statistically analyzed, starting with the general statistical estimators (the statistical mean, the standard deviation of the mean; standard deviation, the variant and the variant coefficient)[4]. Then the Fischer (F) and Tukey (W) values were measured [4] to test the statistical implications of the differences between the eleven parameters, that were the object of the measurements and calculus done for this article, for the five secondary muscle bundles (SMB) taken into study. For the statistical calculus the ANOVA Single Factor (ASF) algorithm (integrated in the Microsoft Excel software) was used.

RESULTS AND DISCUSSIONS

In fish the somatic muscles are structured in muscle fibers of the fourth order (quaternary); third order (tertiary); second order (secondary) and first order (primary). The latter contain a variable number of myocytes or muscle fibers. In the current study, five SMF were investigated from the lateral – dorsal epaxial muscle (ML-ED) which contain 3 to 5 PMFs. For these SMB the parameters taken into study are presented in table 1 through 5. The diameters (big, small and medium), the PMF perimeter, the myocyte number, the transversal section surface of the PMF and their profile were determined. The variability of the raw data was not accentuated for SMF1 (v=6-15%) and SMF2 (v=7-29%), but the specific coefficient (v %) is greater for the other SMFs (tab.3, 4, 5). Table 6 presents the statistical analysis made for the 5 SMFs. Thereby, the big diameter of the PMF from the studied muscle has values between 300 and 675μ, with an average of 477.5±25.212μ (v=22.4%). The small diameter values of the PMB range between 195 and 360μ, with an average of 272.5±11.456μ (v=17.84%) (tab.6). The average thickness values of the primary muscle bundles ranged from 277.5μ to 517.5μ and the average of the 18 primary data was of 375±16.122μ (v=18.24%). The perimeter of these PMBs varied between 871.792μ and 1625.774μ and the statistical mean value was 1178.097±50.65μ (v=18.24%) (tab.6). Regarding the shape index, the variation limits are broad (1.176/1,respectively 2.500/1), with an average of1.779/1 (v=21.67%). The profile index has an average of 58.96±3.22%. These data imply that the PMF from each of the five studied SMB have a cylindroid aspect with a tendency towards flattening.

Table 1.

Statistical indicators for some morphological parameters of the first muscular fascicle ofthe *Polyodon spathula* lateral muscle

Specification					MU	n	Statistical indicators			Variation limits	
Species	Age	Sex	Muscle	Parameter studied at PMF level			±s	s	V(%)	Min.	Max.
Polyodon spathula	The fourth summer (ps 3+)	Male	Dorsal epaxial muscle	Large diameter (LD)	µ	3	575±36,055	62,450	10,86	525	645
				Small diameter (Sd)	µ	3	330±15	25,981	7,87	315	360
				Average diameter (D)	µ	3	452,5±17,5	30,311	6,70	420	480
				PMF* perimeter	µ	3	1421,571	95,224	6,70	1319,469	1507,964
				Format index (If)	x/1	3	1,752±0,152	0,264	15,04	1,542/1	2,048/1
				Profile index (Ip)	%	3	57,90±4,74	8,218	14,19	48,837	64,865
				Number of myocytes	n	3	72,33±2,96	5,132	7,09	68	78
				Transversal section surface of PMF	μ^2	3	148793,682±9485,2	16428,755	11,04	129885,221	159573,272

*PMF = primary muscular fascicle

Table 2.

Statistical indicators for some morphological parameters of the second muscular fascicle ofthe *Polyodon spathula* lateral muscle

Specification					MU	n	Statistical indicators			Variation limits	
Species	Age	Sex	Muscle	Parameter studied at PMF level			±s	s	V(%)	Min.	Max.
Polyodon spathula	The fourth summer (ps 3+)	Male	Dorsal epaxial muscle	Large diameter (LD)	µ	5	399±28,3	63,285	15,86	300	450
				Small diameter (Sd)	µ	5	237±16,02	35,812	15,11	195	285
				Average diameter (D)	µ	5	318±11,275	25,212	7,93	277,5	345
				PMF* perimeter	µ	5	999,027±35,421	79,205	7,93	871,792	1083,849
				Format index (If)	x/1	5	1,735±0,213	0,476	27,43	1,176/1	2,308/1
				Profile index (Ip)	%	5	61,533±8,036	17,969	29,20	43,333	85,00
				Number of myocytes	n	5	34,2±1,685	3,768	11,02	30	38
				Transversal section surface of PMF	μ^2	5	73407,24±4775,1	10677,422	14,55	60082,96	84823,00

*PMF = primary muscular fascicle

Table 3.

Statistical indicators for some morphological parameters of the third muscular fascicle ofthe *Polyodon spathula* lateral muscle

Specification					MU	n	Statistical indicators			Variation limits	
Species	Age	Sex	Muscle	Parameter studied at PMF level			±s	s	V(%)	Min.	Max.
Polyodon spathula	The fourth summer (ps 3+)	Male	Dorsal epaxial muscle	Large diameter (LD)	µ	3	545±78.102	135.277	24.82	405	675
				Small diameter (Sd)	µ	3	310±26.46	45.826	14.78	270	360
				Average diameter (D)	µ	3	427.5±51.96	90.000	21.05	337.5	517.5
				PMF* perimeter	µ	3	1343.031±163.242	282.743	21.05	1060.288	1625.774
				Format index (If)	x/1	3	1.742±0.121/1	0.210	12.04	1.500/1	1.875/1
				Profile index (Ip)	%	3	58.02±4.33	7.500	12.93	53.333	66.667
				Number of myocytes	n	3	59.333±10.10	19.036	32.08	41	79
				Transversal section surface of PMF	μ^2	3	135834.612± 30407.46	52667.272	38.77	85883.289	190851.754

*PMF = primary muscular fascicle (B=bundle)

The number of PMF myocytes varies a lot (v=34.38%) and the variation limits are far from one another (30-79) and the statistical mean for this parameter is 48.444±3.925 mf (tab.6). From the total number of muscle fibers (mf) (myocytes), on average 71.89% were counted (with variations from 42.65% to 100%) (tab.6), which offers credibility and precision for the data that can be analyzed from this characteristic (density and muscle tissue ratio.). The transversal section surface of the PMF varied between 60082.96 μ² and 190851.754 μ², with a statistical mean of 104016.17±8833.73μ² (v=36.03%) (tab.6).

Table 4.

Statistical indicators for some morphological parameters of the fourth muscular fascicle ofthe *Polyodon spathula* lateral muscle

Specification					MU	n	Statistical indicators			Variation limits	
Species	Age	Sex	Muscle	Parameter studied at PMF level			±s	s	V(%)	Min.	Max.
Polyodon spathula	The fourth summer (ps 3+)	Male	Dorsal epaxial muscle	Large diameter (LD)	μ	4	476.25±42.59	85.184	17.89	405	600
				Small diameter (Sd)	μ	4	236.25±15.462	30.923	13.09	195	270
				Average diameter (D)	μ	4	356.25±24.78	49.561	13.91	300	420
				PMF* perimeter	μ	4	1119.192±77.85	155.699	13.91	942.478	1319.469
				Format index (If)	x/1	4	2.030±0.178	0.355	17.51	1.667	2.500/1
				Profile index (Ip)	%	4	50.37±2.111	8.446	16.77	40	60
				Number of myocytes	n	4	43.5±5.38	10.755	24.72	30	54
				Transversal section surface of PMF	μ^2	4	88843.26±10672.7	21345.374	24.03	62026.820	113097.335

*PMF = primary muscular fascicle

Table 5.

Statistical indicators for some morphological parameters of the fifth muscular fascicle ofthe *Polyodon spathula* lateral muscle

Specification					MU	n	Statistical indicators			Variation limits	
Species	Age	Sex	Muscle	Parameter studied at PMF level			±s	s	V(%)	Min.	Max.
Polyodon spathula	The fourth summer (ps 3+)	Male	Dorsal epaxial muscle	Large diameter (LD)	μ	3	445±77.621	134.443	30.21	360	600
				Small diameter (Sd)	μ	3	285±8.66	15.000	5.26	270	300
				Average diameter (D)	μ	3	365±35	60.62	16.61	330	435
				PMF* perimeter	μ	3	1146.682±109.96	190.449	16.61	1036.726	1366.593
				Format index (If)	x/1	3	1.579±0.323	0.560	35.44	1.200/1	2.222/1
				Profile index (Ip)	%	3	68.11±11.75	20.347	29.87	45.0	83.33
				Number of myocytes	n	3	44±7.0	12.124	27.56	37	58
				Transversal section surface of PMF	μ^2	3	98665.645±14286.71	24745.301	25.08	83939.429	127234.503

*PMF = primary muscular fascicle

Table 6.

Statistical indicators for some morphological parameters of the all five secundary muscular fascicles of the *Polyodon spathula* lateral muscle

Specification					MU	n	Statistical indicators			Variation limits	
Species	Age	Sex	Muscle	Parameter studied at PMF level			±s	s	V(%)	Min.	Max.
Polyodon spathula	The fourth summer (ps 3+)	Male	Dorsal epaxial muscle	Large diameter (LD)	µ	18	477.5±25.212	106.967	22.40	300	675
				Small diameter (Sd)	µ	18	272.5±11.456	48.606	17.84	195	360
				Average diameter (D)	µ	18	375±16.122	68.401	18.24	277.5	517.5
				PMF* perimeter	µ	18	1178.097±50.65	214.887	18.24	871.792	1625.774
				Format index (If)	x/1	18	1.779±0.091	0.385	21.67	1.176/1	2.500/1
				Profile index (Ip)	%	18	58.958±3.217	13.648	23.15	40	85
				Total number of myocytes from PMF	n	18	48.444±3.925	16.653	34.38	30	79
				Number of myocytes measured from PMF	n	18	33.444±2.201	9.338	27.92	20	54
				Proportion of myocytes measured from their total number	%	18	71.89±3.61	15.307	21.29	42.65	100.00
				Transversal section surface of PMF	μ^2	18	104016.169±8833.733	37478.353	36.03	60082.96	190851.754

*PMF = primary muscular fascicle

Regarding the thickness and the density of the myocytes from the 18 PMFs, the data presented in table 7 shows that these myocytes have an average diameter of 43.502±0.493µ and a transversal section surface of 1486.523±34.848µ².

Table 7.

Statistical indicators for myocytes density and the proportion of the two tissue categories from primary muscle fascicles of lateral muscle of *Polyodon spathula* fish

Specification						MU	n	Statistical indicators			Variation limits	
Species	Age	Sex	Muscle	Morphological parameters of myocytes and of PMF*					s	V (%)	Min.	Max.
Polyodon spathula fish	Fourth summer	Male	Lateral muscle (Epaxial muscle)	In the myocytes	Large diameter	µ	18	51.641±0.644	2.732	5.29	46.099	56.071
					Small diameter	µ	18	35.361±0.515	2.186	6.18	31.662	39.680
					Average diameter	µ	18	43.502±0.493	2.092	4.81	38.835	47.146
					Cross-sectional area	μ^2	18	1486.523±34.848	147.847	9.95	1177.553	1770.941
				In the PMF	Myocytes density (m.f.**/mm^2)	mf/ mm^2	18	468.911±9.12	38.690	8.25	405.054	551.374
					Muscle tissue proportion	%	18	69.565±1.835	7.787	11.19	57.558	81.782
					Connective tissue proportion	%	18	30.435±1.835	7.786	25.58	18.218	42.442

*PMF=Primay Muscle Fascicle (B=Bundle), **m.f.=muscle fibers (myocytes)

Table 8.

Average statistical values for the thickness and profile of primary muscle fascicles as well as for the density of myocytes and for muscle tissue proportion and connective tissue proportion in lateral muscle of *Polyodon spathula* fish

Specification		Secundary muscle fascicles studied for lateral muscle (average statistical values)					Average value of the five SMF** studied
Morphological parameters studied for PMF*	MU	SMF_1	SMF_2	SMF_3	SMF_4	SMF_5	
Large diameter (LD)	μ	575.0	399.0	545.0	476.25	445.0	**488.05**
Small diameter (Sd)	μ	330.0	237.0	310.0	236.25	285.0	**279.65**
Average diameter (D)	μ	452.5	318.0	427.5	356.25	365.0	**283.85**
PMF perimeter (P)	μ	1421.571	999.027	1343.031	1119.192	1146.682	**1205.9006**
Format index of PMF	x/1	1.752/1	1.735/1	1.742/1	2.030/1	1.579/1	**1.7676/1**
Profile index of PMF	%	57.90	61.533	58.02	50.37	68.11	**59.1866**
Total number of myocytes from PMF	n	72.33	34.20	59.333	43.50	44	**50.6726**
Cross-sectional area from PMF	μ^2	148793.682	73407.240	135834.612	88843.260	98665.645	**109108.8878**
Density of myocytes from PMF	mf***/mm^2	488.265	470.248	444.952	489.166	444.282	**467.3826**
Proportion of muscle tissue from PMF	%	64.098	74.998	65.070	71.376	68.059	**68.7202**
Proportion of connective tissue from PMF	%	35.902	25.002	34.930	28.624	31.941	**31.2798**

*PMF=Primary Muscle Fascicle, **SMF=secundary muscle fibers (myocytes), ***m.f.=muscle fibers (myocytes).

The myocyte density varies between 405.054 mf/mm² and 551.374 mf/mm² and the statistical mean for this characteristic is 468.911±9.12mf/mm² (v=8.25%) (tab.7). For the pure muscle tissue ratio in the 18 PMFs, the values ranged between 57.558% and 81.782%, with a statistical mean of 69.565±1.835% (v=11.19%) (tab.7).The conjunctive tissue ratio was on average of 30.435±1.835% (tab.7). The average values of the studied parameters (for the PMFs) where then compared between the 5 SMFs, as shown in table 8. Also in table 8 the arithmetic mean of the 5 SMFs was calculated for each of the 11 parameters taken into study. It can be observed that the big diameter values of the PMF vary between 399μ and 575μ and the average of the 5 SMF is 488.05μ.

Table 9.

Statistical significance of the differences between the five (5) secundary muscular fascicles of the lateral muscle of *Polyodon spathula*, regarding the thickness and profile of myocytes from PMF.

Studied parameters of PMF* from SFM**	Differences between average values of the 5 SMF compared	Tukey values(W'=0,01)	Statistical significance	At 4; 13 LL (liberty levels), for:			
				P	p≤0.05	p≤0.01	p≤0.001
				Fα	3.180	5.200	9.070
Large diameter of PMF(μ)	SMF_1-SMF_2= 176,0	235,3117	n.s.		2,092000		
	SMF_1-SMF_3=30,0	306,3163	n.s.				
	SMF_1-SMF_4=98,75	311,9512	n.s.				
	SMF_1-SMF_5=130,00	353,8695	n.s.				
	SMF_2-SMF_3=146,00	235,3116	n.s.				
	SMF_2-SMF_4=77,25	251,6648	n.s.				
	SMF_2-SMF_5=46,00	298,2824	n.s.				
	SMF_3-SMF_4=68,75	246,0948	n.s.				
	SMF_3-SMF_5=100,00	306,3163	n.s.				
	SMF_4-SMF_5=31,25	246,3491	n.s.				
Small diameter of PMF (μ)	SMF_1-SMF_2=93,00	106,9252	n.s.		6,074270		
	SMF_1-SMF_3=20,00	139,1896	n.s.				

	SMF_1-SMF_4=93,75	141,7501	n.s.		
	SMF_1-SMF_5=45,00	160,7976	n.s.		
	SMF_2-SMF_3=73,00	106,9252	n.s.		
	SMF_2-SMF_4=0,75	114,356	n.s.		
	SMF_2-SMF_5=48,00	135,539	n.s.		
	SMF_3-SMF_4=73,75	111,825	n.s.		
	SMF_3-SMF_5=25,00	139,1896	n.s.		
	SMF_4-SMF_5=48,75	111,8251	n.s.		
Average diameter of PMF (μ)	SMF_1-SMF_2=134,50	150,472	n.s.		4,073117
	SMF_1-SMF_3=25,00	195,8765	n.s.		
	SMF_1-SMF_4=69,25	199,478	n.s.		
	SMF_1-SMF_5=87,50	226,2847	n.s.		
	SMF_2-SMF_3=109,50	150,4720	n.s.		
	SMF_2-SMF_4=38,25	160,9292	n.s.		
	SMF_2-SMF_5=47,00	190,7391	n.s.		
	SMF_3-SMF_4=71,25	157,3674	n.s.		
	SMF_3-SMF_5=62,50	195,8765	n.s.		
	SMF_4-SMF_5=8,75	157,3674	n.s.		
Perimeter of PMF (μ)	SMF_1-SMF_2=422,544	472,7215	n.s.		4,073123
	SMF_1-SMF_3=78,540	615,3639	n.s.		
	SMF_1-SMF_4=302,379	626,6839	n.s.		
	SMF_1-SMF_5=274,889	710,8942	n.s.		
	SMF_2-SMF_3=344,004	472,7215	n.s.		
	SMF_2-SMF_4=120,165	505,5736	n.s.		
	SMF_2-SMF_5=147,655	599,2244	n.s.		
	SMF_3-SMF_4=223,839	494,384	n.s.		
	SMF_3-SMF_5=196,349	615,3639	n.s.		
	SMF_4-SMF_5=27,490	494,384	n.s.		
Format index of PMF (Fi)(x/1)	SMF_1-SMF_2=0,017	0,8478	n.s.		0,588310
	SMF_1-SMF_3=0,010	1,1036	n.s.		
	SMF_1-SMF_4=0,278	1,1239	n.s.		
	SMF_1-SMF_5=0,173	1,2749	n.s.		
	SMF_2-SMF_3=0,007	0,8478	n.s.		
	SMF_2-SMF_4=0,295	0,9067	n.s.		
	SMF_2-SMF_5=0,156	1,0747	n.s.		
	SMF_3-SMF_4=0,288	0,8866	n.s.		
	SMF_3-SMF_5=0,163	1,1036	n.s.		
	SMF_4-SMF_5=0,451	0,8876	n.s.		
Profile index of PMF (Pi)(%)	SMF_1-SMF_2=3,633	30,023	n.s.		0,737192
	SMF_1-SMF_3=0,120	39,0823	n.s.		
	SMF_1-SMF_4=7,530	39,8012	n.s.		
	SMF_1-SMF_5=10,210	45,1495	n.s.		
	SMF_2-SMF_3=3,513	30,023	n.s.		
	SMF_2-SMF_4=11,163	32,1094	n.s.		
	SMF_2-SMF_5=6,577	38,0573	n.s.		
	SMF_3-SMF_4=7,650	31,3988	n.s.		
	SMF_3-SMF_5=10,090	39,0823	n.s.		
	SMF_4-SMF_5=17,74	31,3987	n.s.		

Table 10.

Statistical significance of the differences between the five (5) secundary muscular fascicles of the lateral muscle of *Polyodon spathula*, regarding the number and density of myocytes and the proportion of
muscular and connective tissue from PMF

Studied parameters of PMF* from SFM**	Differences between average values of the 5 SMF compared	Tukey values(w=0,01)	Statistical significance	At 4; 13 LL (liberty levels), for:			
				P	p≤0.05	p≤0.01	p≤0.001
				Fα	3.180	5.200	9.070
Total number of myocytes from PMF	SMF_1-SMF_2=38,130	36,634	**		7,13688144		
	SMF_1-SMF_3=12,997	47,6883	n.s.				
	SMF_1-SMF_4=28,830	48,5655	n.s.				
	SMF_1-SMF_5=28,330	55,0915	n.s.				
	SMF_2-SMF_3=25,133	36,634	n.s.				
	SMF_2-SMF_4=9,300	39,1800	n.s.				

	SMF_2-SMF_5=9,800	46,4375	n.s.		
	SMF_3-SMF_4=15,833	38,3128	n.s.		
	SMF_3-SMF_5=15,333	47,6883	n.s.		
	SMF_4-SMF_5=0,500	38,3128	n.s.		
Cross section of PMF (μ^2)	SMF_1-SMF_2=75386,442	82446,961	n.s.		5,245366
	SMF_1-SMF_3=12959,070	107325,111	n.s.		
	SMF_1-SMF_4=59950,422	109299,426	n.s.		
	SMF_1-SMF_5=50128,037	123986,469	n.s.		
	SMF_2-SMF_3=62427,372	82446,961	n.s.		
	SMF_2-SMF_4=15436,020	88176,683	n.s.		
	SMF_2-SMF_5=25258,405	104510,232	n.s.		
	SMF_3-SMF_4=46991,352	86225,103	n.s.		
	SMF_3-SMF_5=37168,967	107325,111	n.s.		
	SMF_4-SMF_5=9822,385	86225,232	n.s.		
Density of myocytes from PMF (m.f./mm^2)***	SMF_1-SMF_2=18,017	89,9886	n.s.		0,927429
	SMF_1-SMF_3=43,313	117,1425	n.s.		
	SMF_1-SMF_4=0,901	119,2974	n.s.		
	SMF_1-SMF_5=43,983	135,3279	n.s.		
	SMF_2-SMF_3=25,296	89,9886	n.s.		
	SMF_2-SMF_4=18,918	96,2425	n.s.		
	SMF_2-SMF_5=25,966	114,0701	n.s.		
	SMF_3-SMF_4=44,214	94,1124	n.s.		
	SMF_3-SMF_5=0,670	117,1425	n.s.		
	SMF_4-SMF_5=44,884	94,1124	n.s.		
Proportion of muscular tissue (MT)(%) from PMF	SMF_1-SMF_2=10,90	17,1293	n.s.		1,448594
	SMF_1-SMF_3=0,972	22,2980	n.s.		
	SMF_1-SMF_4=7,278	22,7082	n.s.		
	SMF_1-SMF_5=3,961	25,8945	n.s.		
	SMF_2-SMF_3=9,928	17,1293	n.s.		
	SMF_2-SMF_4=3,622	18,3197	n.s.		
	SMF_2-SMF_5=6,939	21,7132	n.s.		
	SMF_3-SMF_4=6,306	17,9143	n.s.		
	SMF_3-SMF_5=2,989	22,2980	n.s.		
	SMF_4-SMF_5=3,317	17,9143	n.s.		
Proportion of connective tissue (CT)(%) from PMF	SMF_1-SMF_2=10,90	17,1293	n.s.		1,448594
	SMF_1-SMF_3=0,972	22,2980	n.s.		
	SMF_1-SMF_4=7,278	22,7082	n.s.		
	SMF_1-SMF_5=3,961	25,8945	n.s.		
	SMF_2-SMF_3=9,928	17,1293	n.s.		
	SMF_2-SMF_4=3,622	18,3197	n.s.		
	SMF_2-SMF_5=6,939	21,7132	n.s.		
	SMF_3-SMF_4=6,306	17,9143	n.s.		
	SMF_3-SMF_5=2,989	22,2980	n.s.		
	SMF_4-SMF_5=3,317	17,9143	n.s.		

*PMF=Primary Muscle Fascicle, **SMF=secundary muscle fibers (myocytes), ***m.f.=muscle fibers (myocytes).

The small diameter values are between 236.25µ and 330µ, with a mean of 279.65µ (tab.8). The average thickness of the 5 SMFs is 383.85µ, with limits between 318µ and 452.5µ. The PMF perimeter values from the 5 SMFs ranges from 999.027µ to 1421.671µ (tab.8). The shape index values range from 1.579/1 to 2.030/1, with a mean of 1.768/1, which highlights an accentuated cylindroid aspect (shape) of the SMF. This is confirmed also by the values of the profile index (Ip=50.37-68.11%; Ip=59.19%) (tab.8). Regarding the total number of myocytes, it varied between 34.20 and 72.33mf/mm², with a mean for the 5 SMFs of 50.67mf/mm². For the transversal section surface of the PMF, the mean for the 5 SMF was of 109108.888µ², with values between 73407.24µ² and 148793.682µ² (tab.8). The myocyte density varied less in the 5 SMFs, with values ranging from 444.282mf/mm² to 489.166mf/mm² and the arithmetic mean was 467.383mf/mm² (tab.8). The muscle tissue ratio in the SMF varied between 64.098% and 74.998%, with a mean for the 5 SMFs of 68.72%

(tab.8). The conjunctivetissue ratio values ranged from 25% to 35.90%, with a mean for the 5 SMFs of 31.28% (tab.8). The existing differences for the five secondary muscle bundles (regarding the 11 parameters analyzed) are notable, with smaller or bigger values, yet these differences are not significant from a statistical point of view. Thus, for the small, average and big diameter values of the PMB, the F values are lower than Fα=0,01 at 4;13 GL, and Tukey (w'=0,01) values are greater than the tested differences (tab. 9). The same can be observed regarding the differences between the perimeters; shape indices; profile indices; the transversal section surfaces; myocyte densities and the muscle and conjunctive tissue ratios (tab.9) (tab.10). Only in the case of the myocyte number values, the difference between SMF1 and SMF2 was statistically significant (F>Ftab.α=0,01, at 4;13 GL) (w'<dif.test.) (tab.10). Regarding the thickness of the myocytes from the lateral-dorsal epaxial muscle of Polyodon Spathula, the values found are comparable with the ones obtained by other authors [7], yet smaller than for other species of fish, for example trout (47-67µ). Also, the pure muscle tissue ratio found in the studied muscle is greater in *Polyodon spathula*, when compared to other species of animals (chicken, pork, and beef). The small ratio of conjunctive tissue from the analyzed muscle, positively correlates with the reduced percentage of collagen in this meat, which in Ps3+ fish was of 4.21% [7], determining a higher tenderness to the meat from this species of fish.

CONCLUSIONS

1. The average thickness of the primary muscle bundles (PMF), from 4 year old Polyodon Spathula lateral-dorsal epaxial muscle was of 383.85µ.
2. The perimeter of these structural muscle units (PMF), for the species and for the studied muscle is 1205.9µ.
3. The primary muscle bundles (PMF) from this muscle have an accentuated cylindroid shape (If=1,768/1) (Ip=59,19%).
4. The myocyte number of the PMF, in the studied muscle is 50.67mf, for the four year old Polyodon Spathula (Ps3+).
5. The transversal section surface of the PMF from the studied muscle (ML-ED) is 109108.888µ².
6. The PMF myocytes have an average thickness of 43.502µ; a transversal section surface of 1486.523µ² and a density of 467.38mf/mm².
7. The pure muscle tissue ratio, for the Polyodon Spathula species, in the lateral-dorsal epaxial muscle is of 68.72% and positively correlates with the myocyte density.
8. The conjunctive tissue ratio for the same species and muscle is 31.28% and it correlates positively with the low ratio of collagen (4.21%) from the meat obtained from Polyodon Spathula.

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